## Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells.

**Journal:** Nature

**Publication Year:** 2014

**Authors:** Johanna Flach, Sietske T Bakker, Mary Mohrin, Pauline C Conroy, Eric M Pietras, Damien Reynaud, Silvia Alvarez, Morgan E Diolaiti, Fernando Ugarte, E Camilla Forsberg, Michelle M Le Beau, Bradley A Stohr, Juan Mendez, Ciaran G Morrison, Emmanuelle Passegue

**PubMed link:** 25079315

**Funding Grants:** Mechanisms Underlying the Responses of Normal and Cancer Stem Cells to Environmental and Therapeutic Insults, UCSC Shared Stem Cell Facility, Training Program in Stem Cell Research at UCSF, UCSC Shared Stem Cell Facility

**Public Summary:**
This study provides important new insights into the mechanics of HSC aging. HSCs self-renew for life, thereby making them one of the few blood cells that truly age. Paradoxically, although HSCs numerically expand with age, their functional activity declines over time, resulting in degraded blood production and impaired engraftment following transplantation. While many drivers of HSC aging have been proposed, the reason why HSC function degrades with age remains largely unknown. Here, we show that cycling old HSCs have heightened levels of replication stress associated with cell cycle defects and chromosome gaps/breaks, which are due to decreased expression of mini-chromosome maintenance (MCM) DNA helicase components and altered dynamics of DNA replication forks. Nonetheless, old HSCs survive replication unless confronted with a strong replication challenge like treatment with replication stressor drugs in vitro or transplantation in vivo. Moreover, once old HSCs re-establish quiescence, residual replication stress on ribosomal DNA (rDNA) genes leads to the formation of nucleolar-associated γH2AX signals, which persist mainly due to ineffective H2AX de-phosphorylation by mislocalized PP4c phosphatase rather than ongoing DNA damage. Persistent nucleolar γH2AX also acts as a histone modification marking the transcriptional silencing of rDNA genes and decreased ribosome biogenesis in quiescent old HSCs. Our results identify replication stress as a potent driver of functional decline in old HSCs, and highlight the MCM DNA helicase as a potential molecular target for rejuvenation therapies. They also suggest a novel non-canonical function for γH2AX as an epigenetic histone modification that marks the silencing of the transcription machinery, which could be an important mechanism to block transcription in genomic regions undergoing DNA repair. It will now be important to determine whether decreased rDNA gene transcription in quiescent old HSCs plays a role in BM failure syndromes and other age-related blood defects linked to defective ribosome function. This article was previewed in Nature (512:140-141, 2014), and selected for Faculty of 1000 Biology.

**Scientific Abstract:**
Haematopoietic stem cells (HSCs) self-renew for life, thereby making them one of the few blood cells that truly age. Paradoxically, although HSCs numerically expand with age, their functional activity declines over time, resulting in degraded blood production and impaired engraftment following transplantation. While many drivers of HSC ageing have been proposed, the reason why HSC function degrades with age remains unknown. Here we show that cycling old HSCs in mice have heightened levels of replication stress associated with cell cycle defects and chromosome gaps or breaks, which are due to decreased expression of mini-chromosome maintenance (MCM) helicase components and altered dynamics of DNA replication forks. Nonetheless, old HSCs survive replication unless confronted with a strong replication challenge, such as transplantation. Moreover, once old HSCs re-establish quiescence, residual replication stress on ribosomal DNA (rDNA) genes leads to the formation of nucleolar-associated gammaH2AX signals, which persist owing to ineffective H2AX dephosphorylation by mislocalized PP4c phosphatase rather than ongoing DNA damage. Persistent nucleolar gammaH2AX also acts as a histone modification marking the transcriptional silencing of rDNA genes and decreased ribosome biogenesis in quiescent old HSCs. Our results identify replication stress as a potent driver of functional decline in old HSCs, and highlight the MCM DNA helicase as a potential molecular target for rejuvenation therapies.

haematopoietic